MIN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In re Application of:	)	Conf. No.: 3910
Beka SOLOMON	)	Art Unit: 1649
Appln. No.: 09/441,140	)	Examiner: G. S. Emch
Filed: November 16, 1999	)	Washington, D.C.
For: PREVENTION OF PROTEIN AGGREGATION	)	

DECLARATION OF JONATHAN M. GERSHONI UNDER 37 C.F.R. §1.132

Honorable Commissioner for Patents
U.S. Patent and Trademark Office
Randolph Building, Mail Stop Amendments
401 Dulany Street
Alexandria, VA 22314

Sir:

I, the undersigned Jonathan M. Gershoni, hereby declare and state as follows.

I am a Full Professor of Cell Research of Immunology and incumbent of the David Furman chair in Immunobiology of Cancer at Tel Aviv University. I have been studying protein:protein interactions for the last 30 years and especially the antibody:antigen interaction. This research has entailed the production of polyclonal antibodies in mice and rabbits as well as isolation of thousands of murine monoclonal

antibodies against synthetic peptides, natural proteins, recombinant proteins and peptides as well as phage displayed peptides. In addition to isolation and characterization of antibodies, I have developed numerous solid phase immunoassays as well as novel computational algorithms for the specific purpose of mapping and characterizing conformational discontinuous epitopes. The main goal of my research over the last 20 years has been the rational design of effective peptide immunogens as vaccines. My Curriculum Vitae is submitted herewith.

I have been asked to review the publication Gaskin et al., "Human Antibodies Reactive with β-Amyloid Protein in Alzheimer's Disease," *J. Exp. Med.* 177-1181-1186 (1993). I have been informed that, because Gaskin reports that each of the isolated monoclonal antibodies binds to Aβ 1-28, the examiner has taken the position that each of the four antibodies isolated by Gaskin would necessarily be obtainable using Aβ 1-28 as an immunogen. I have been asked whether, in light of my review of the Gaskin publication and my knowledge of the state of the art, I would agree or disagree with that position of the examiner. My response and reasoning, as supported by the literature in the field, are set out below.

Gaskin discovered four rare mAbs (see the paragraph bridging pages 1184-1185 of Gaskin), produced *in vivo*, that bind Aβ 1-28 yet show more efficient binding to Aβ 1-40, as demonstrated in a competition assay. While the Gaskin paper states that the significance of the paper is in that it is a first illustration of autoimmunity against Aβ protein, the examiner uses Gaskin to suggest that these unique antibodies could be generated using Aβ 1-28 peptide alone. The examiner's argument is, effectively, that as the mAbs bind Aβ 1-28, this segment should suffice as an effective immunogen stimulating the production of antibodies that behave as do those isolated by Gaskin, i.e., are identical to those isolated by Gaskin. For the reasons explained below, this could hypothetically be possible, but it is highly unlikely.

The hypothetical scenario - Aβ 1-28 is sufficient.

In order for Aβ 1-28 to suffice as an immunogen, then one would have to explain the enhanced binding to Aβ 1-40. This could be done based on the argument that all of the contacts are in linear Aβ 1-28 yet Aβ 29-40 cause the peptide to aggregate, thus generating a multivalent complex that can bind Ab with increased avidity. Two points might support this: (1) Gaskin argues that aggregation might be the cause of break of tolerance

required to elicit the mAbs (see the last sentence of the first full paragraph of page 1185), and (2) residues 29-40 tend to be hydrophobic while all the charged and polar residues reside in 1-28.

While this is a theoretically possible explanation, it is not the most likely.

The identification of a peptide bound by a given mAb does not promise that immunization with said peptide leads to reproduction of antibodies similar to the original mAb. A case in point is the mAb 2F5 that binds the linear sequence ELDKWAS of HIV gp41. This mAb is highly cross-neutralizing and thus numerous labs have sought to generate immunogens that will reproduce similar neutralizing activity. Many papers have been published illustrating attempts to use the linear peptide and its variations as a vaccine. Submitted herewith are two such papers: Coëffier et al., "Antigenicity and immunogenicity of the HIV-1 gp41 epitope ELDKWA inserted into permissive sites of the MalE protein," *Vaccine* 19:684-693 (2001); and Ho et al., "Conformational constraints imposed on a pan-neutralizing HIV-1 antibody epitope result in increased antigenicity but not neutralizing response," *Vaccine* 23:1559-1573 (2005). In most of the publications, anti-peptide activity is easily produced.

However, the antibodies produced often do not even cross-react with the native antigen, namely gp41 or gp160 (which contains gp41), and most certainly never create antibodies that are efficient in cross-neutralization of HIV. Thus, identification of a core peptide recognized by a mAb most often does not imply that the peptide will be sufficient as an immunogen to reproduce that antibody.

This inability to reproduce the original antibody is even more likely when the original antibodies are the product of natural events (as opposed to using synthetic peptides in adjuvant). The natural antigen in Gaskin was the full length Aβ protein. Moreover, these antibodies are the result of rare autoimmune reactions for which a unique cascade of events may be necessary to present the epitope such that it can break tolerance. One should not expect that Aβ 1-28 would be able to reproduce the unique circumstances that the full length protein was able to break tolerance *in vivo* in an autologous situation.

Therefore, the most likely explanations for Gaskin's result of enhanced binding for 1-40 are based on two lines of thought that are basic and common in the field of antibody:epitope interaction, both of which exclude the possibility that 1-28 would be sufficient.

1. The sequence Aβ 29-40 contributes some genuine contacts with the mAbs. Thus, the core binding is to Aβ 1-28 and a given affinity is realized. However, there is enhanced binding to Aβ 1-40 via contacts postulated to exist in residues 29-40. Thus, the simplest explanation is that the antibodies bind residues in residues 1-28 and 29-40 of Aβ. There are numerous examples where antibodies bind discontinuous residues; in fact, this is the norm (see Rubinstein et al., "Computational characterization of B-cell epitopes," *Mol Immunol.* 45(12):3477-89 (2008), of which I am a co-author, a copy of which is submitted herewith). The question is whether such an antibody would be able to detectably bind partial segments of the epitope alone. The answer is definitively yes.

It is common practice in standard pepscan analyses to detect clear binding to multiple and distant peptides, illustrating the discontinuous nature of the epitope being analyzed (see, for example, Lundkvist et al., "Mapping of B-Cell Epitopes in the Nucleocapsid Protein of Puumala Hantavirus, *Viral Immunology*, 15:177-192 (2002), a copy of which is submitted herewith). Note Figure 4 in Lundkvist, which shows the results of a pepscan that is a collection of 141 peptides 14 amino acids long and a pitch of 3 amino acids, i.e., they each overlap 11 amino acids of the following or preceding peptide.

One can see patterns where the antibody binds strongly to a preferred peptide, but also binds more weakly to the overlapping peptides on either side of the preferred one. Furthermore, as illustrated in Figure 4B, the mAb 2E12 detectably binds to 2 discontinuous clusters of peptides, one around sequence 52-65 while the other around residues 142-158 (see also Figure 5). Obviously, both regions of the antigen would be necessary to faithfully reproduce a mAb similar to 2E12 and neither 52-65 nor 142-158 would suffice on its own. If the Gaskin antibodies require residues in Aβ 29-40, as is implicated by the preferred binding to residues 1-40 vs 1-28, then it would be impossible to precisely reproduce the Gaskin antibodies using Aβ 1-28 alone.

2. The second argument would be that residues 29-40 are essential for imposing a unique but critical conformation in residues 1-28, which residues 1-28 could not otherwise assume alone. Thus, one would argue that all the contacts may reside in residues 1-28, yet the conformation of those contacts was dependent on interactions with residues 29-40. Indeed as described in Sgourakis et al., "The Alzheimer's peptides Aβ40 and 42 adopt distinct conformations in water: A combined MD / NMR study," *J Mol Biol*, 368(5):1448-1457 (2007),the sequence LVFF (see point #3 in the paragraph bridging pages 2 and 3 of the Author Manuscript submitted herewith) may likely be involved

in interaction with the C-terminal aspect of 1-40 and thus there is support for a novel conformation of residues 1-28, dependent on 29-40.

Obviously, the combination of both 1 and 2 is also possible.

Thus, based on the facts that:

1. the natural immunogen was the full length Aβ,

2. the immunization was naturally autologous and involved unique circumstances of break of tolerance,

3. residues 29-40 are reported to impose conformational constraints on residues 1-28 of Aβ, and

4. often more than a core peptide is required to generate complex antibody activities (such as the case of 2F5), I would conclude that it would be highly unlikely to expect that the Aβ 1-28 synthetic peptide would be able to elicit antibodies similar to those reported by Gaskin. Accordingly, I definitely could not conclude that, based on the information provided in the Gaskin publication, at least one of the four disclosed antibodies of Gaskin would necessarily be producible using Aβ 1-28 is the immunogen. In my opinion, for the reasons provided above, such a conclusion would be unsupportable.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made

on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

December 17, 2008 /Jonathan M. Gershoni/

Date Jonathan M. Gershoni